

DISCOVERY OF A NEW POPULATION OF *JUNIPERUS GRACILIOR* VAR. *URBANIANA* FROM THE DOMINICAN REPUBLIC: ANALYSES OF LEAF TERPENOIDS AND SNPS FROM nrDNA AND trnC-trnD

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ABSTRACT

A new population of *Juniperus gracilior* var. *urbaniana* is reported from the Dominican Republic (DR). Previously, the taxon has been known from only one location on the slopes of Pic La Selle, Haiti. The new DR population is not typical of the variety and differs in a few SNPs as well as some differences in its volatile leaf oil. The new population of *J. g.* var. *urbaniana* may be of hybrid origin with *J. g.* var. *ekmanii*. *Phytologia* 92(3): 413-423 (December 1, 2010).

KEY WORDS: *Juniperus gracilior* var. *urbaniana*, *J. g.* var. *gracilior*, *J. g.* var. *ekmanii*, Cupressaceae, leaf terpenoids, nrDNA, trnC-D, taxonomy.

Juniperus gracilior var. *urbaniana* (Pilger & Ekman) R. P. Adams is known from only a single population on Pic La Selle, Haiti, where it grows as a prostrate plant in the pine forest on an unusual

white, chalky soil (Adams, 2008). Recently, shrubby junipers were discovered in the Parque Nacional Siera de Bahoruco (Baoruco), Dominican Republic (DR), near the Haitian border. Because the determination of these juniper varieties is very difficult based only on morphology, sequencing of nrDNA and trnC-trnD was performed along with analyses of the volatile leaf oils to more precisely determine the relationship of the shrubby junipers to *J. gracilior* var. *urbaniana* from Haiti, *J. g.* var. *ekmanii* (Florin) R. P. Adams, Haiti and *J. g.* var. *gracilior* Pilger, Dominican Republic.

MATERIALS AND METHODS

Specimens collected: taxon, acronym, collector number, location: *J. barbadensis* (BA), Adams 5367-5371; Petit Piton, St. Lucia, BWI; *J. bermudiana* (BM), Adams 11080-11082, Bermuda; *J. gracilior* var. *ekmanii* (EK), Adams 7653-7654, 3-4 km ne Mare Rouge, Pic la Selle, Haiti; *J. gracilior* var. *gracilior* (GR), Adams 7664-7667, w of Constanza, Dominican Republic, Adams 3097-3105, Pedernales, DR; *J. gracilior* var. *urbaniana* (UR) Adams 7656-7658, 4-5 km ne Mare Rouge, Pic la Selle, Haiti, Jimenez 4160(3), (= Adams 12005, 12006, 12314 at BAYLU), Parque Nacional Siera de Bahoruco (Baoruco), N 18° 14' 57", W 71 ° 37' 53", 2100 m; *J. lucayana*: Adams 5259-5280, Havana Botanical Garden (seed from Sierra de Nipe), Cuba; Adams 5281-5282, Havana Botanical Garden (seed from Isle de Pinos), Cuba; *J. saxicola* (SX) Adams 5284-5285, w slope of Pico Turquino, Prov. Granma/ Santiago de Cuba boundary, Cuba; *J. virginiana* var. *virginiana* (VG) Adams 6753-6755; on hwy. 135, Hewitt, TX; *J. virginiana* var. *silicicola* (SI) Adams 9186-9188, Ft. Desoto Park, Mullet Key, Florida. Herbarium vouchers are deposited at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR amplification ITS (nrDNA), trnC-trnD amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (trnC-trnD) or

K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μ M each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 μ M each primer. See Adams, Schwarzbach and Morris (2008) for the nrDNA and trnC-trnD primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (S. San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Compositional analyses of the volatile leaf oils of all the taxa in Hispanola are shown in Table 1. Notice that the oil of the shrub from DR is dominated by bornyl acetate as are all the taxa from DR. The oils of *J. g. var. urbaniana* appear to share only a few unique compounds: elemol, unknown 1611, β -eudesmol and α -eudesmol. The oils of *J. g. var. ekmanii* and *var. urbaniana* also share some unique compounds: δ -2-carene, isoborneol, germacrene D, piperitone and methyl eugenol. Although some of these are found in trace amounts in the *J. g. var. gracilior* plants from Pedernales (gracP in Table 2). The oils of *J. g. var. urbaniana* from DR and Haiti also have some quantitative differences: sabinene (4.5, 12.8), borneol (10.7, 1.6) and bornyl acetate (38.1, 26.2). But overall, it appears that the leaf oil of the DR shrubs are most similar to *var. urbaniana* from Haiti. The oil

from putative *J. g.* var. *gracilior* from Pedernales (near the shrubby junipers) is similar to both *J. g.* var. *gracilior*, Constanza and *J. g.* var. *ekmanii* (Table 1) in sharing several unique compounds: unknowns 900 and 907 and linalool. But the oil also has some compounds in common with *J. g.* var. *urbaniana* and *J. g.* var. *ekmanii* (but not *J. g.* var. *gracilior*): trans-sabinene hydrate, citronellol, methyl eugenol, epicubebol and trans-cadina-1,4-diene, suggesting the Pedernales population is intermediate between *J. g.* var. *gracilior* and *J. g.* var. *ekmanii*.

Sequencing nrDNA revealed 23 SNPs (Single Nucleotide Polymorphisms) among the Caribbean taxa. A minimum spanning network shows that the DR shrub (Ud, Fig. 1) is separated from *J. g.* var. *urbaniana*, Haiti (Uh, Fig. 1) by 2 SNP differences. Notice that *J.*

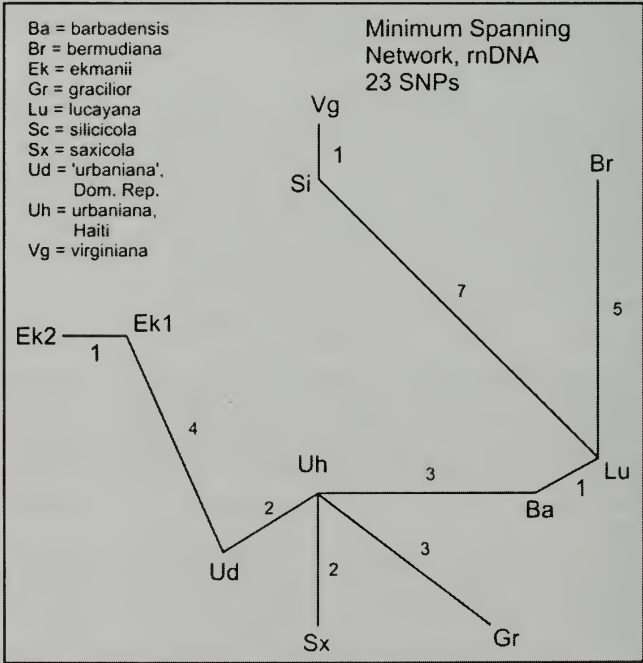


Figure 1. Minimum spanning network using 23 nrDNA SNPs for the Caribbean junipers.

saxicola is linked by 2 SNPs (Fig. 1) to *J. g. var. urbaniana*, Haiti (Uh, Fig. 1). It is interesting that Ud is intermediate to *J. ekmanii* (Ek1, Ek2).

Analyses of trnC-trnD sequences revealed no differences between the DR shrub and *J. g. var. gracilior*, and one difference between it and *J. g. var. urbaniana* (Haiti) and *J. g. ekmanii* (Fig. 2). Integrating these data with previous data (Adams, et al. 2008) shows (Fig. 2) that the 'urbaniana' shrubs from Dominican Republic are in the group with *J. g. var. gracilior*, whereas *J. g. var. urbaniana* (Haiti) is in a group with *J. g. var. ekmanii* and *J. saxicola*. However, these groups are separated by only one SNP.

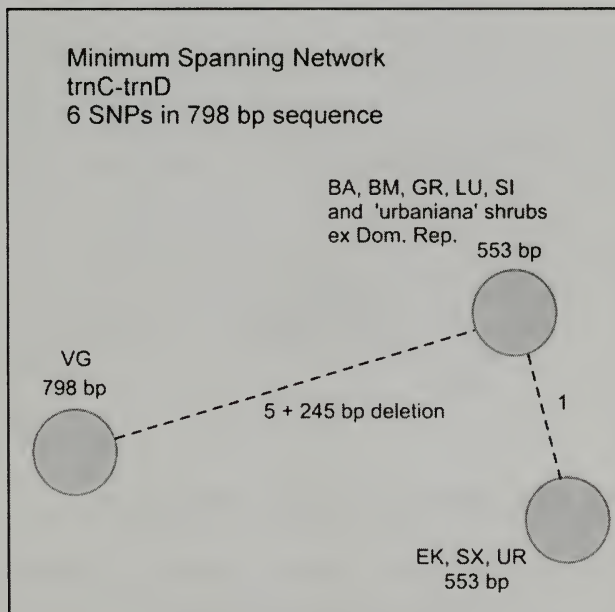


Figure 2. Minimum Spanning Network based on 6 SNPs from trnC-trnD 798 bp sequence.

CONCLUSIONS

It is noteworthy that a new population of *J. g. var. urbaniana* has been discovered. This new site is in the same mountain range as Pic La Selle and represents an eastern extension of the range (Fig. 3). The new population differs somewhat from the Haiti plants in that these shrubs are about 0.3 m tall x 1-2 m wide, whereas the plants in Haiti are prostrate (5-10 cm tall x 3-5 m wide). The DR shrubs appear to grow on a hard-pan type of soil. The DR plants differ somewhat in their DNA sequences and volatile leaf oils that suggests possible hybridization with *J. g. var. ekmanii*. Nevertheless, it is still useful for conservation purposes to have this new population, because the Haitian population is very small and certainly threatened.



Figure 3. Distribution map for *Juniperus gracilior var. urbaniana*.

ACKNOWLEDGMENTS

This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for lab assistance. Thanks to R. García, director of Jardín Botánico Nacional, Brigido Peguero, Alberto Veloz & Rosa Rodríguez for their valuable help in field work.

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Table 1. Comparisons of the volatile leaf oils of *J. gracilior* var. *urbaniana*, shrub from Dominican Republic (urbD), *J. g.* var. *urbaniana*, Haiti (urbH), *J. g.* var. *ekmanii* (ekman), *J. g.* var. *gracilior*, Constanza, DR (gracC) and *J. g.* var. *gracilior*, Pedernales, DR (gracP). Compounds in bold appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. RI is the Kovat's Index using a linear approximation on DB-5 column.

RI	compound	urbD	urbH	ekman	gracC	gracP
900	unknown, <u>43,55,125,140</u>	-	-	t	0.2	0.3
907	unknown, <u>43,55,125,140</u>	-	-	t	0.2	0.3
921	tricyclene	1.9	1.0	1.3	3.2	0.9
924	α -thujene	0.4	0.6	0.3	0.7	0.7
932	α -pinene	3.0	1.6	3.3	3.7	2.1
946	camphene	1.9	1.2	1.5	2.5	1.2
969	sabinene	4.5	12.8	10.1	4.4	11.8
974	β -pinene	0.2	0.4	0.4	.04	0.3
988	myrcene	2.6	3.2	2.5	2.2	3.0
1001	δ-2-carene	0.2	0.6	0.2	-	-
1002	α -phellandrene	0.1	t	t	t	0.1
1008	δ -3-carene	-	-	-	t	-
1014	α -terpinene	0.9	2.4	0.7	1.1	1.3
1020	p-cymene	1.4	2.8	0.4	2.6	1.0
1024	limonene	6.5	9.0	8.4	7.9	7.0
1025	β -phellandrene	3.0	5.0	4.2	3.8	3.6
1054	γ -terpinene	2.0	2.8	1.4	3.3	2.6
1065	cis-sabinene hydrate	0.5	1.0	0.6	0.1	0.8
1086	terpinolene	0.6	1.0	0.5	0.6	0.7
1090	6,7-epoxymyrcene	-	t	t	t	0.1
1098	trans-sabinene hydrate	0.3	0.4	0.3	-	0.4
1098	linalool	t	-	0.6	0.1	0.6
1100	n-nonanal	t	t	-	-	-
1112	trans-thujone	0.1	0.1	0.7	0.6	0.2
1118	cis-p-menth-2-en-1-ol	0.3	0.4	0.4	0.2	0.5
1118	cis-limonene oxide	-	-	0.1	-	-
1136	trans-p-menth-2-en-1-ol	0.1	0.4	0.1	0.1	0.4
1141	camphor	1.2	1.6	3.4	0.7	0.8

RI	compound	urbD	urbH	ekman	gracC	gracP
1145	camphene hydrate	1.0	0.6	1.3	0.4	0.6
1154	karahanaenone	-	-	-	0.1	-
1155	isoborneol	0.2	0.2	0.1	-	t
1165	borneol	10.7	1.6	3.3	1.1	1.7
1167	umbellulone	0.2	t	t	t	t
1174	terpinen-4-ol	3.2	4.8	3.7	3.3	7.4
1178	naphthalene	-	0.2	-	-	-
1184	dill ether	-	-	0.1	0.2	0.3
1186	α-terpineol	1.4	1.0	0.3	0.2	0.4
1191	cis-dihydrocarvone	-	-	t	t	-
1195	cis-piperitol	-	-	-	t	-
1195	methyl chavicol	-	t	-	-	0.3
1200	trans-dihydrocarvone	-	-	t	-	-
1207	trans-piperitol	-	t	-	-	0.5
1223	citronellol	-	-	0.3	-	0.4
1232	thymol, methyl ether	-	-	-	t	-
1239	carvone	t	-	t	t	t
1241	carvacrol, methyl ether	-	-	-	-	-
1249	piperitone	0.8	0.8	0.3	-	t
1274	pregeijerene B	-	0.2	-	-	-
1284	bornyl acetate	38.1	26.2	37.6	46.2	33.0
1287	safrole	-	-	-	-	2.6
1298	carvacrol	-	-	-	r	-
1299	terpin-4-yl acetate	t	-	-	-	t
1326	iso-dihydrocarvyl acetate	-	-	-	0.1	-
1345	α -cubebene	0.1	-	-	-	-
1356	neo-iso-dihydrocarvyl acetate	-	-	-	0.2	-
1365	cis-carvyl acetate	-	-	-	0.1	-
1374	α -copaene	0.2	-	-	-	-
1380	daucene	-	-	0.2	-	-
1387	β -bourbonene	t	-	-	-	-
1387	β -cubebene	0.1	-	t	-	-
1396	duvalene acetate	-	-	-	-	t
1403	methyl eugenol	1.2	0.6	0.1	-	2.2
1401	α -cedrene	-	-	t	-	-
1413	β -funebrene	-	-	t	-	-

RI	compound	urbD	urbH	ekman	gracC	gracP
1417	(E)-caryophyllene	0.3	t	t	0.4	-
1429	cis-thujopsene	-	-	t	0.1	-
1448	cis-muurola-3,5-diene	0.4	0.2	0.1	0.1	0.1
1452	α -humulene	0.2	t	-	t	-
1475	trans-cadina-1(6),4-diene	0.4	0.2	0.1	0.2	0.1
1478	γ -muurolene	-	-	t	t	-
1480	germacrene D	0.5	0.2	t	-	-
1493	trans-muurola-4(14),5-diene	1.1	0.6	0.1	0.2	0.4
1493	epi-cubebol	0.1	t	0.1	-	0.2
1500	α -muurolene	0.2	t	0.1	t	-
1513	cubebol	0.4	0.4	0.6	t	0.5
1522	δ -cadinene	0.9	0.4	0.6	0.5	0.3
1528	zonarene	0.3	0.2	0.1	0.2	0.1
1530	dauca-4(11),8-diene	-	-	t	-	-
1532	γ -cuparene	-	-	-	t	-
1533	trans-cadina-1,4-diene	0.1	t	t	-	t
1537	α -cadinene	-	-	t	-	-
1544	α -calacorene	-	-	-	t	-
1548	elemol	0.1	0.8	-	-	-
1555	elemicin	-	t	t	0.4	2.5
1574	germacrene-D-4-ol	-	-	0.8	-	-
1582	caryophyllene oxide	t	-	t	0.1	0.2
1587	trans-muuro-5-en-4- α -ol	t	t	t	-	t
1599	widdrol	-	-	t	t	-
1600	cedrol	-	t	0.3	0.2	-
1607	β -oplopenone	t	-	0.3	-	t
1608	humulene epoxide II	-	-	-	t	t
1611	unknown,43,109,119,254?	0.3	0.6	-	-	-
1627	1-epi-cubenol	0.5	0.6	0.4	0.5	0.6
1630	γ -eudesmol	-	0.1	-	-	-
1638	epi- α -cadinol	t	t	0.2	0.1	-
1638	epi- α -muurolol	t	t	0.2	0.1	-
1644	α -muurolol	t	t	t	t	-
1645	cubenol	-	-	-	-	0.2
1649	β-eudesmol	t	0.4	-	-	-
1652	α-eudesmol	t	0.4	-	-	-

RI	compound	urbD	urbH	ekman	gracC	gracP
1652	α-cadinol	-	-	0.4	-	-
1685	germacra-4(15),5,10(14)-trien-1-al	t	t	t	t	0.3
1887	oplopanonyl acetate	-	-	t	-	-
1905	isopimara-9(11),15-diene	t	-	-	-	t
2055	abietatriene	t	t	-	-	t
2087	abietadiene	-	-	-	-	0.1
2282	sempervirol	0.3	0.4	-	0.2	0.6
2314	trans-totarol	0.3	0.4	0.4	2.0	0.5
2331	trans-ferruginol	t	t	-	0.1	t